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In vivo imaging studies of cytisine

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ABSTRACT

Cytisine, a plant alkaloid available as an effective treatment for smoking dependence, is used to target the nicotinic acetylcholine receptor (nAChR). Direct detection of nAChR localization *in vivo* in the brain can help to understand the mechanism of cytisine antismoking action and improve clinical treatment. However, visualization of cytisine targeting *in vivo* requires suitable techniques for assaying molecular interactions noninvasively. For use in clinical imaging techniques, the cytisine molecule requires chemical modification to be visualized *in vivo*. Herein, we review the current development of modified cytisine probes for detection using positron emission tomography (PET), single photon emission computed tomography (SPECT) and computed tomography (CT). The detection of cytisine using magnetic resonance imaging (MRI) is also discussed.

KEYWORDS: *cytisine, nicotinic acetylcholine receptors, Magnetic Resonance Imaging, Positron Emission Tomography, monitoring.*

1. INTRODUCTION

Advances in *in vivo* clinical techniques such as positron emission tomography (PET), single photon emission computed tomography (SPECT), computed tomography (CT) and magnetic resonance imaging (MRI) can provide internal images of neuronal structure and function. The clinical techniques (PET, SPECT, CT, MRI) can be used to obtain images before and after treatment to evaluate drug localization and efficiency in diseased tissue. These techniques provide high-resolution and high-sensitivity detection. To obtain images using PET, SPECT and CT, radiotracer drugs radiolabeled with markers have to be synthesized. The basic requirements for a radiotracer are (1) rapid preparation; (2) binding affinity for the targeted receptor; (3) penetration through the intact blood–brain barrier; and (4) efficient *in vivo* accumulation. Detection of the distribution of drugs *in vivo* remains a major difficulty. The appearance of the same molecules in healthy and diseased brain tissues makes discrimination difficult. In addition to the clinical techniques mentioned above, magnetic resonance imaging (MRI) has been gaining in popularity for the detection and imaging of *in vivo* drug targeting, bio-distribution and drug metabolism. MRI has offered additional tools for drug discovery and development, evaluation of pharmacokinetic properties and monitoring drug efficacy. *In vivo* imaging of drug delivery, release and subsequent monitoring of the therapeutic outcomes can greatly aid and enhance treatment. Our interest is in the imaging of cytisine targeting of the nicotinic acetylcholine receptor (nAChR) which is a ligand-gated ion channel crucial to normal and diseased brain physiology. The nAChR is a therapeutic target in a wide range of pathological conditions such as Parkinson's disease, neuropathic pain and nicotine addiction [1]. Using PET, CT or SPECT *in vivo*, cytisine ligands of the nAChR have to be labeled with radiotracers such as carbon-11 or fluorine-18. The syntheses of cytisine radioligands is time sensitive and must be accomplished without radioactive dilution with stable isotope. In this review, insights in nAChR targeting with radiolabeled cytisine is discussed. Briefly, cytisine, an alkaloid from the plant *Laburnum anagyroides Med*., (*Cytisus laburnum L., Fabaceae*), has been classified as a selective, low-efficacy partial agonist of $\alpha_4\beta_2$ subunit of the nAChR [2,3]. Several syntheses of radiolabeled cytisine derivatives for image monitoring of distribution and targeting has been achieved and are described in this review.

2. EXPERIMENTAL AND METHODS

The research discussed here reflects the use of cytisine and *in vivo* imaging techniques. The data was collected from the PubMed database.

3. RESULTS: *IN VIVO* IMAGING STUDIES

The nicotinic subunit $\alpha_4\beta_2$ of nAChR expressed in the human brain was imaged and reported using PET and SPECT [4]. A nine α (α_2–α_{10}) and three β (β_2–β_4) subunits have been identified that are distributed throughout the central nervous system (CNS) [4,5]. Among the several nAChR subtypes in the CNS, the homomeric α_7 and heteromeric $\alpha_4\beta_2$ subtypes are predominant in the brain. These subtypes are best characterized in terms of their ligand selectivity and they can be studied by means of binding techniques: [^{3}H]cytisine or [^{3}H]nicotine can label $\alpha_4\beta_2$nAChR, and [^{125}I]α-bungarotoxin or [^{3}H]methyllycaconitine is used to label α_7nAChR. Cytisine is a partial agonist of the $\alpha_4\beta_2$ nAChR subtype and has been used as a tritiated radioligand to probe nAChR function. The current known radiotracer probes are ^{18}F-2-FA85380 for PET [6,7] and ^{123}I-5-A85380 for SPECT [8,9]. Following intravenous injection of N-[^{11}C] methylcytisine (Figure 1), uptake in a baboon brain was studied and showed an uptake

concentration different from blood radioactivity. *In vivo* binding of [^{11}C] appeared different from *in vivo* binding of [^{3}H] cytosine [10]. 9-(fluorophenyl)cytisine derivatives were obtained in a Suzuki coupling of 4-fluorobenzeneboronic acid and 9-bromo-N-Boc cytisine [11, 12, 13]. Decarbonylation of this labeled aldehyde with Wilkinson catalyst afforded 4-[^{18}F] fluorobromobenzene. As compared to homoaromatic and aliphatic nucleophic radiofluorinations, nucleophilic substitution of a nitro group by a fluoride [^{18}F] in the pyridine series appears to be a highly efficient method for the synthesis of ^{18}F-radiotracers of high specific activity [14]. The cytisine radiotracer was formed in one step from the corresponding nitro derivative. Nitropyridine [15] and 9-(2-fluoro-5-pyridinyl)cytisine [16], were prepared by a Stille coupling of iodocytisine [17]. Another reaction to generate radiotracer was cytisine with fluoroiodobenzene [18]. Allain Barbier and coworkers demonstrated the possibility of carrying out the reaction of cytisine with 4-[^{18}F]fluorobromobenzene (Figure 2) [19].

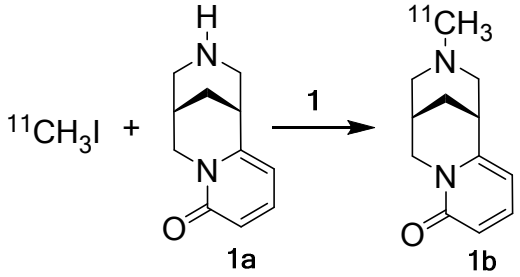

Figure 1. Radiosynthesis of [^{11}C] Methylcytisine (1b) using cytisine (1a).

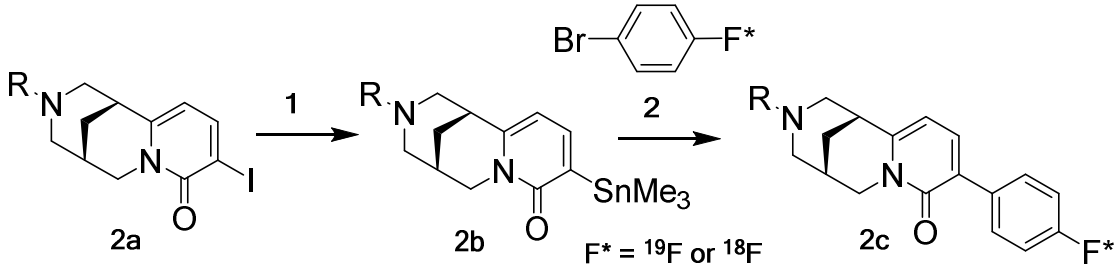

Figure 2. Synthesis of 9-(4-[^{18}F]fluoropyridinyl) cytisine (2c) where R = an aliphatic group**.**

The limited number of *in vivo* MRI papers related to drug delivery to the brain is surprising low with respect to the broad availability and capability of this *in vivo* method. In a study by Chefer and coworkers, cytisine (1 mg/kg) was subcutaneously injected into the blood stream and binding of cytisine to the nAChR was detected by MRI which imaged the spatial distribution of cytisine in brain [20]. Using co-registered MRI/PET images, Allen et. al (1997) identified densities of nAChR in the brain. The uptake of radiotracer was detected and showed dysfunction of brain using MRI [21]. Abnormalities of the nAChR in autism were also detected using the radioligand, 6-chloro-3-((2-(S)-azetidinyl)methoxy)-5-(2-fluoropyridin-4-yl) pyridine using PET/MRI imaging *in vitro* and *in vivo*. Estimated binding potential values in different brain regions which characterize the specificity of receptor binding of radiotracer have been provided [22]. In another study, nAChR occupancy in the human brain was detected using varenicline (0.5 mg) which is a derivative of cytisine, commonly prescribed for smoking cessation. This finding demonstrates that a low dose of varenicline saturates $\alpha_4\beta_2$nAChR in the human brain [23]. Functional mapping using imaging techniques such as functional magnetic resonance imaging (fMRI) measure the hemodynamic response of the brain in relation to drug activity [24]. In another study, decreased acetylcholine levels in ^{3}H-cytisine-labeled nicotinic $\alpha_4\beta_2$ receptors was studied by PET and MRI [25]. The binding of [^{3}H]cytisine in rat brain homogenates was examined and showed 60-90% total binding at all concentrations examined up to 15 nM. The nicotinic cholinergic agonists nicotine, acetylcholine, and carbachol compete with high affinity for [^{3}H]cytisine binding sites, whereas among nicotinic receptor antagonists only dihydro-beta-erythroidine competes with high affinity. Comparison of binding in several brain regions showed that [^{3}H]cytisine binding is higher in the thalamus, striatum, and cortex than in the hippocampus, cerebellum, or hypothalamus. The pharmacology and brain regional distribution of [^{3}H]cytisine binding sites are those predicted for neuronal nicotinic receptor agonist recognition sites[25]. The high affinity and low nonspecific binding of [^{3}H]cytisine makes it a useful ligand for studying nAChR.

4. CONCLUSIONS

This review has shown that cytisine is molecule with a unique and synthetically and functionally challenging structure which can bind to the $\alpha_4\beta_2$ subunit of nAChR. During the past century, in particular in the last two decades, enormous progress has been made in understanding the chemistry of cytisine. The introduction of radionuclei to the structure of cytisine markedly improved the monitoring of drug *in vivo*.

5. REFERENCES

[1] Bencherif M., Neuronal nicotinic receptors as novel targets for inflammation and neuroprotection: mechanistic considerations and clinical relevance, *Acta Pharmacologica Sinica*, 30, 702–714, **2009.**

[2] Lukas R.J., Pharmacological effects of nicotine and nicotinic receptor subtype pharmacological profiles. In: George TP, editor. Medication treatments for nicotine dependence. CRC Press, Boca Raton, 3-23, **2006.**

[3] Tutka P., Zatoński W., Cytisine for the treatment of nicotine addiction: from a molecule to therapeutic efficacy, *Pharmacological Reports*, 58, 777–798, **2006.**

[4] Bauwens M., Mottaghy F.M., Bucerius J., PET Imaging of the Human Nicotinic Cholinergic Pathway in Atherosclerosis, *Current Cardiology Reports,* 17, 8, 67, **2015.**

[5]Posadas I., López-Hernández B., Ceña V., Nicotinic Receptors in Neurodegeneration, *Current Neuropharmacology,*11, 3, 298–314, **2013.**

[6] Kimes A.S., Horti A.G., London E.D., Chefer S.I., Contoreggi C., Ernst M., 2-[^{18}F]F-A-85380: PET imaging of brain nicotinic acetylcholine receptors and whole body distribution in humans, *Journal of the Federation of American Societies for Experimental Biology*, 17, 1331–1333, **2003.**

[7] Bottlaender M., Valette H., Roumenov D., Dolle F., Coulon C., Ottaviani M., Hinnen F., Ricard M., Biodistribution and radiation dosimetry of ^{18}F-fluoro-A-85380 in healthy volunteers, *Journal of Nuclear Medicine*, 44, 596– 601, **2003.**

[8] Fujita M., Ichise M., van Dyck C.H., Zoghbi S.S., Tamagnan G., Mukhin A.G., Bozkurt A., Seneca N., Tipre D., DeNucci C.C., Iida H., Vaupel D.B., Horti A.G., Koren A.O., Kimes A.S., London E.D., Seibyl J.P., Baldwin R.M., Innis R.B., Quantification of nicotinic acetylcholine receptors in human brain using ^{123}I-5-IA85380 SPET, *European Journal of Nuclear Medicine*, 30, 1620–1629, **2003.**

[9] Chefer S.I., London E.D., Koren A.O., Pavlova O.A., Kurian V., Kimes A.S., Horti A.G., Mukhin A.G., Graphical analysis of 2-[18F]FA binding to nicotinic acetylcholine receptors in rhesus monkey brain, *Synapse,* 48, 25–34, **2003.**

[10] Valette H., Bottlaender M., Dollé F., Dolci L., Syrota A., Crouzel C., An attempt to visualize baboon brain nicotinic receptors with N-[^{11}C]ABT-418 and N-[^{11}C]methyl-cytisine, *Nuclear Medicine Communications*, 18, 164, **1997.**

[11] van Tamelen E.E., Baren J.S., The synthesis of dl-cytisine, *Journal of American Chemical Society*, 77, 4944-4945, **1955.**

[12] Smrčiná M., Lorenc M., Hanus V., Sedmerá P., Kočovsky P.J., Synthesis of enantiomerically pure 2,2'-dihydroxy-1,1'-binaphthyl, 2,2'-diamino-1,1'-binaphthyl, and 2-amino-2'-hydroxy-1,1'-binaphthyl. Comparison of processes operating as diastereoselective crystallization and as second order asymmetric transformation, *Journal of Organic Chemistry,* 57, 1917-1920, **1992.**

[13] Zhang Y., Yeung S.M., Wu H., Heller D.P., Wu C., Wulff W.D., Highly enantioselective deracemization of linear and vaulted biaryl ligands, *Organic Letters*, 5, 1813-1816, **2003.**

[14] Lasne M.C., Perrio C., Rouden J., Barré L., Roeda D., Dollé F., Crouzel C., In Contrast Agent II. Topics in Current Chemistry; Krause, W., Ed.; Springer-Verlag: Berlin Vol. 222, pp 201−258, **2002.**

[15] Campos K.R., Klapars A., Waldman J.H., Dormer P.G., Chen C., Enantioselective, palladium-catalyzed alpha-arylation of N-Boc-pyrrolidine, *Journal of American Chemical Society*, 128, 3538-3539, **2006.**

[16] Barker G., McGrath J.L., Klapars A., Stead D., Zhou G., Campos K.R., O'Brien P.J., Enantioselective, palladium-catalyzed α-arylation of N-Bocpyrrolidine: in situ react IR spectroscopic monitoring, scope, and synthetic applications, *Journal of Organic Chemistry,* 76, 5936-5953, **2011.**

[17] Bao J., Wulff W.D., Dominy J.B., Fumo M.J., Grant E.B., Rob A.C., Whitcomb M.C., Yeung S.M., Ostrander R.L., Rheingold A.L., *Journal of American Chemical Society*, 118, 3392-3405, **1996.**

[18] Germain A.R., Bruggemeyer D.M., Zhu J., Genet C., O'Brien P., Porco J.A., Synthesis of the azaphilones (+)-sclerotiorin and (+)-8-O-methylsclerotiorinamine utilizing (+)-sparteine surrogates in copper-mediated oxidative dearomatization, *Journal of Organic Chemistry*, 76, 2577-2584, **2011.**

[19] Allain-Barbier L., Lasne M.C., Perrio-Huard C., Moreau B., Barré L., Synthesis of 4[F-18] fluorophenylalkenes and arenes via palladium catalyzed coupling of 4-[F-18] fluoroidobenzene with vinyl and aryl tin reagents, *Acta Chemica Scandinavica*, 52, 480-489, **1998.**

[20] Chefer S.I., Horti A.G., Lee K.S., Koren A.O., Jones D.W., Gorey J.G., Links J.M., Mukhin A.G., Weinberger D.R., London E.D., The effects of lobeline on α4β2 nicotinic acetylcholine receptor binding and uptake of [^{18}F]nifene in rats, *Journal of Neuroscience Methods,* 214, 163–169, **2013.**

[21] Allen G., Buxton R.B., Wong E.C., Courchesne E., Attentional activation of the cerebellum independent of motor involvement, *Science*, 275, 1940–1943, **1997.**

[22] Brasic J.R., Zhou Y., Musachio J.L., Hilton J., Fan H., Crabb A., Endres C.J., Reinardt M.J., Dogan A.S., Alexander M., Rousset O., Maris M.A., Galecki J., Nandi A., Wong D.F., Single Photon Emission Computed Tomography (SPECT) Experience with (S)-5-[^{123}I]iodo-3-(2-azetidinylmethoxy)pyridine (5-[^{123}I]IA) in the Living Human Brain of Smokers and Nonsmokers, *Synapse,* 63, 4, 339–358, **2009.**

[23] Lotfipour S., Mandelkern M., Alvarez-Estrada M., Brody A.L., A Single Administration of Low-Dose Varenicline Saturates$\alpha_4\beta_2$ Nicotinic Acetylcholine Receptors in the Human Brain, *Neuropsycho pharmacology*, 37, 1738–1748, **2012.**

[24] Ogawa S., Lee T.M., Kay A.R., Tank D.W., Brain magnetic resonance imagingwith contrast dependent on blood oxygenation, 87, 9868-9872, **1990.**

[25] Kuruvilla S., Mukherjee J., Suppression of acetylcholine breakdown by butyrylcholinesterase inhibitor in postmortem Alzheimer's disease brain, *J Nucl Med,* 55, 416, **2014.**